Genetics and Genetic engineering Dept. **Agric. Biotechnology Program**

Molecular Biology Exam **Level: Three**

First Semester: 2019 / 2020 **Time: 2 Hours**

ANSWER THE FOLLOWING QUESTIONS

First question : (20 Marks)

Write Notes about two point only:

(1): Types of Gene mutations and chromosomal mutations.

(2): Structure and Function of Ribosome in Prokaryote and Eukaryote.

(3): General recombination and site specific recombination. Applications of Recombinant DNA technology..

Second Question : (20 Marks)

Write Notes about :

(1): Mention Types of DNA damage and Explain Two Types of DNA Repair.

(2): Explain the main steps of protein synthesis.

Third Question : (20 Marks)

(1): Compare between Three only:

1.(RNA Polymerases - DNA Polymerases)

2. (A constitutive gene – A housekeeping gene)

3..(Regulator, Structural and Operator genes)

4. (Lac operon and Trp operon in prokaryote.)

(2): Complete The followings: (Only five Points)

(1): DNA replication and Transcription in Prokaryotes occur in---------- While translation occurs in ……..

(2): The main function of DNA is ……

(3): …… Most popular protocol for sequencing DNA, very adaptable, ---------- to large sequencing projects.

(4): A section of bacterial DNA that regulates the transcription of structural genes in an operon ---------- gene.

(5): The code links groups of nucleotides in an ----------to---------- in a----------.

(6): The functions of Transcription factors are ----------

(7): The main steps of gene cloning are ---------,---------,--------- etc.

(8): The most important properties of genetic code are ----------,----------,----------,----------etc.

With my best wishes

Prof. Dr / Mohamed Serag Eldin

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Model Answer

First question : (20 Marks)

Write Notes about two point only :
(1): Types of Gene mutations and chromosomal mutations

Mutation is a change in genetic material.

Gene Mutations:

A. Point Mutation B. Frameshift Mutation

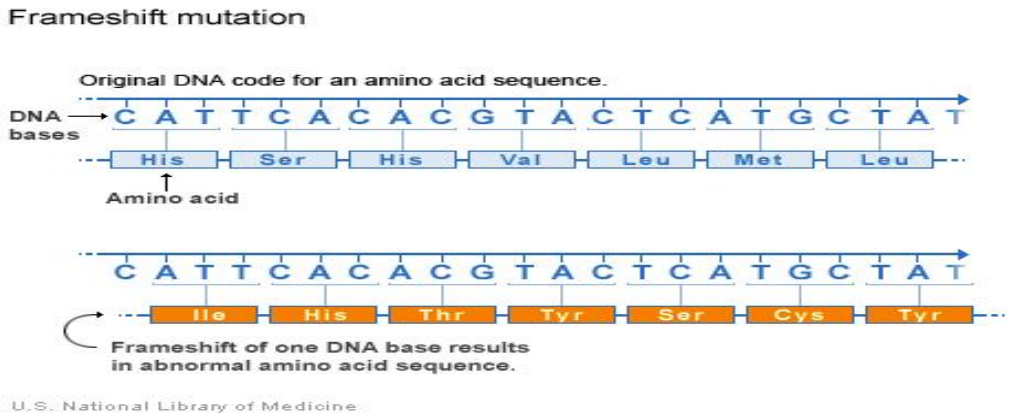
Point Mutation: occur at a single point

Includes substitution, addition, and deletions of bases.

May only change one amino acid coded for.

Frameshift Mutation: when codons get changed because of additions or deletions

Changes the combinations so that different amino acids are coded for.



Chromosomal Mutations

These mutations change the entire chromosome.

Types include:

Deletions: loses part of chromosome

Duplications: doubles part of chromosome

Inversions: inverts part of chromosome

Translocations: takes part of chromosome and moves it to other part of chromosome.

(2): Structure and Function of Ribosome in Prokaryote and Eukaryote.

Eukaryotic Ribosomes

80S particle with 40S and 60S subunits • 40S contains 18S RNA (1874 bases) and 33 polypeptides

60S contains 28S RNA (4718 bases), 5.8S RNA (160 bases), 5S RNA (120 bases) and 49 polypeptides

Mitochondria have ribosomes similar to prokaryotic ribosomes.

prokaryotic ribosomes (E. coli)			
Ribosome	***Subunit***	***rRNAs***	***r-proteins***
70S	***50S***	***23S (2904 nt)*** ***5S (120 nt)***	***31***
	30S	***16S (1542 nt)***	***21***
eukaryotic cytosolic ribosomes (R. norvegicus)s			
Ribosome	***Subunit***	***rRNAs***	***r-proteins***
80S	***60S***	***28S (4718 nt)*** ***5.8S (160 nt)*** ***5S (120 nt)***	***49***
	40S	***18S (1874 nt)***	***33***

The ribosomes present in the prokaryotes function differently in protein production than the ribosomes of the eukaryote organisms. The ribosomes of bacteria, archea and eukaryotes differ significantly from each other in structure and RNA sequences.

The differences in the ribosomes allows the antibiotic to kill the bacterial ribosome by inhibiting the activity of the bacterial ribosomes, the human ribosome sramin unaffected. The ribosomes of the eukaryotic cells are similar to the ribosomes of the bacterial cells, showing the evolutionary origin of the organelle.

Structures of the subunits

50S and 30S besides difference in size, also have somewhat different structures

Both are rather complicated, since they're much larger than average protein

The 50S subunit: This part of ribosome contains site where new peptide bonds are formed when proteins are synthesized

mRNA would be located horizontally in groove across middle

To help with protein synthesis, subunit uses adenine RNA nucleotide, shown by green dot in center

The 30S subunit :

Unlike 50S, 30S is fairly flexible

It needs to have movable regions because, when it shifts from one mRNA nucleotide triplet to another, movement is necessary to aid this process

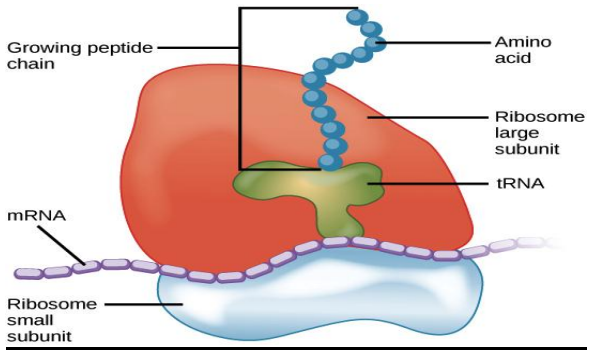
30S controls flow of information during protein synthesis

30S finds an mRNA and then makes sure that each tRNA is matched up correctly on mRNA

It's been suggested that mRNA enters through small hole in 30S (shown here in center of left side

mRNA then extends up into the place where translation occurs, located in cleft between top part, "head", and bottom part, "body

The Purpose of ribosomes:



Ribosome basically a protein factory. Subunits each have role in making of proteins. Ribosomes decode mRNA and form peptide bonds

To understand exactly what each subunit does, it's necessary to walk through protein synthesis step by step

(3): General recombination and site specific recombination. Applications of Recombinant DNA technology..

GENERAL RECOMBINATION

Heteroduplex joint

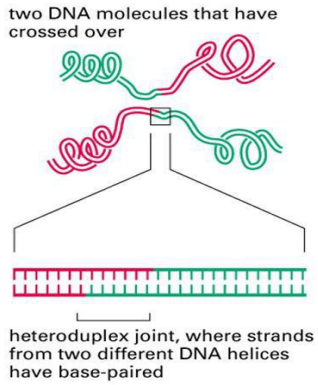


Figure 5–55. Molecular Biology of the Cell, 4th Edition.

Two homologous DNA molecules cross over.

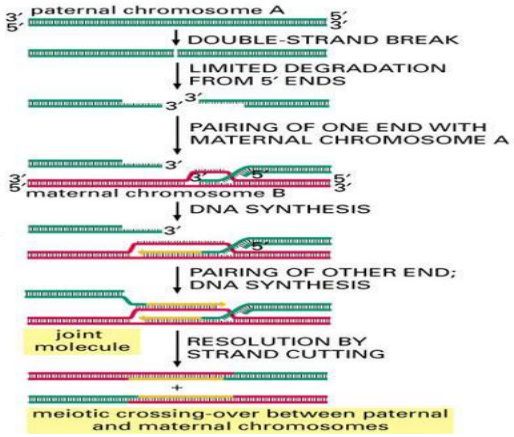
The site of the exchange can occur anywhere.

A strand of one DNA molecule has become base-paired to a strand of the second DNA to create heteroduplex joint.

No nucleotide sequences are altered.

The procedure of general recombination

DNA synapsis: base pairs form between complementary strands from the two DNA molecules



General recombination allows large fraction of genetic information to move from one chromosome to another.

General recombination requires the breakage of double helices, beginning with a single strand breakage.

General recombination is facilitated by Rec A in bacteria and its homologous in eukaryotes.

Holiday junction is the intermediate state of general recombination.

Site Specific Recombination

Moves specialized nucleotide sequence (mobile genetic elements) between non-homologous sites within a genome.

Trans positional site specific recombination.

Conservative site specific recombination.

Three of the many types of mobile genetic elements found in bacteria

Transposase gene: encoding enzymes for DNA breakage and joining
Red segments: DNA sequences as recognition sites for enzymes
Yellow segments: antibiotic genes

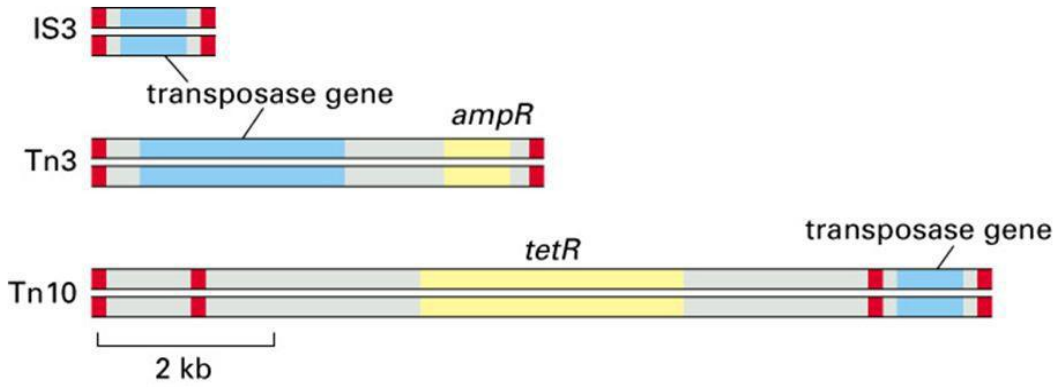


Figure 5–69. Molecular Biology of the Cell, 4th Edition.

Applications of Recombinant DNA Technology

1. Analysis of Gene Structure and Expression
2. Pharmaceutical Products
   - Drugs , Vaccines

3. Genetically modified organisms (GMO)
   - Transgenic plants , Transgenic animal

Second Question : (20 Marks)

Write Notes about :

(1): Mention Types of DNA damage and Explain Two Types of DNA Repair.

Types of DNA Damage

Deamination: (C → U and A→ hypoxanthine

Depurination: purine base (A or G) lost

linked, T-T more prominent, T-T and T-C dimers: bases become cross-light (UV-C (<280 nm) and UV-B (280-320 nm) caused by UV

Alkylation: an alkyl group (e.g., CH3) gets added to bases; chemical induced; some harmless, some cause mutations by mispairing during replication or stop polymerase altogether

Oxidative damage: guanine oxidizes to 8-oxo-guanine, also cause SS and DS breaks, very important for organelles.

Replication errors: wrong nucleotide (or modified) inserted.

Double-strand breaks (DSB): induced by ionizing radiation, transposons, topoisomerases, homing endonucleases, and mechanical stress on chromosomes.

Types of DNA Repair

There are many types of repair: In Light (Photoreactivation) in Dark (Mismatch repair, Base excision repair(BER) , Nucleotide excision Repair (NER) etc..

Repair of UV-induced dimers in the light

Photoreactivation

Light-dependent, UV-A → blue light (360-420 nm).

Catalyzed by Photolyases:

Enzymes that convert the dimers to monomers.

Use FAD as chromophore and electron donor.

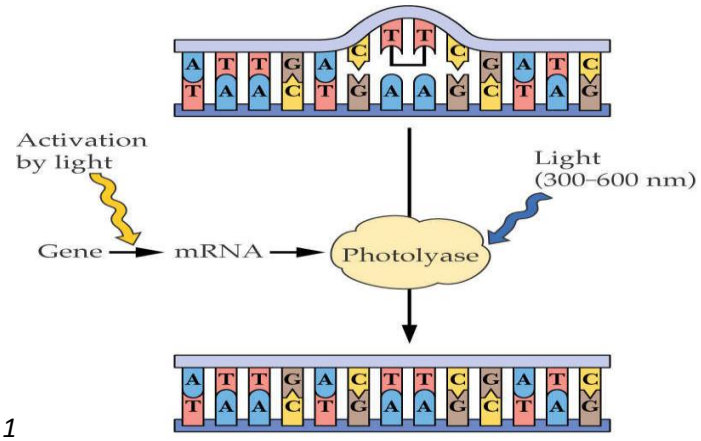
also have another chromophore that acts as antenna

photolyase for T-C 3 classes: CPD I and II for T-T dimers, and a 6-4 dimers

Arabidopsis has CPD II and 6-4 photolyases

and possibly Arabidopsis also has a photolyase in the chloroplast one in the mitochondria.

Photolyase gene expression also induced or increased by light.



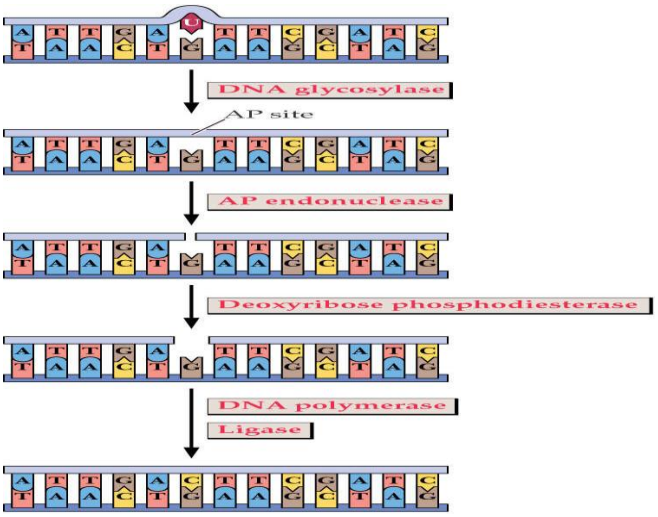
1

Base Excision Repair (BER)

Variety of DNA glycosylases, for different types of damaged bases.

AP endonuclease recognizes sites with a missing base; cleaves sugar-phosphate backbone.

Deoxyribose phosphodiesterase removes the sugar-phosphate lacking the base.



(2): Mention the main steps of protein synthesis.

3 important stages in protein synthesis:

- The coding by triplets of bases to produce mRNA (Transcription)
- The linking of mRNA to tRNA at ribosomes (Translation)
- The linking of amino acids to form polypeptides

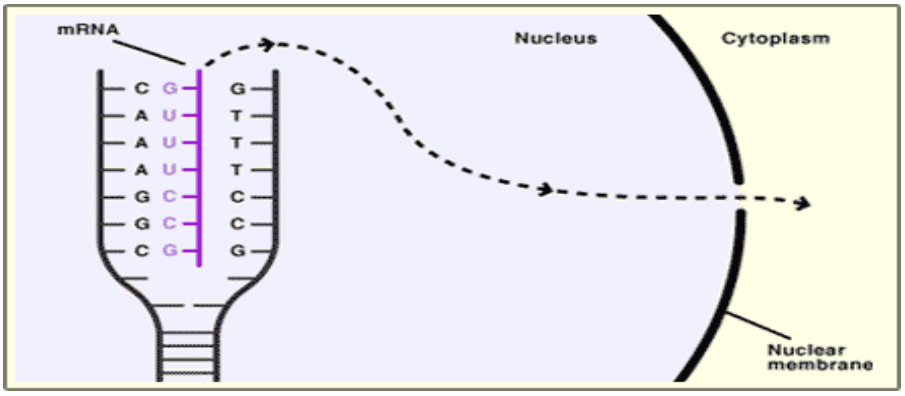
Transcription of mRNA

Transcription proceeds through:

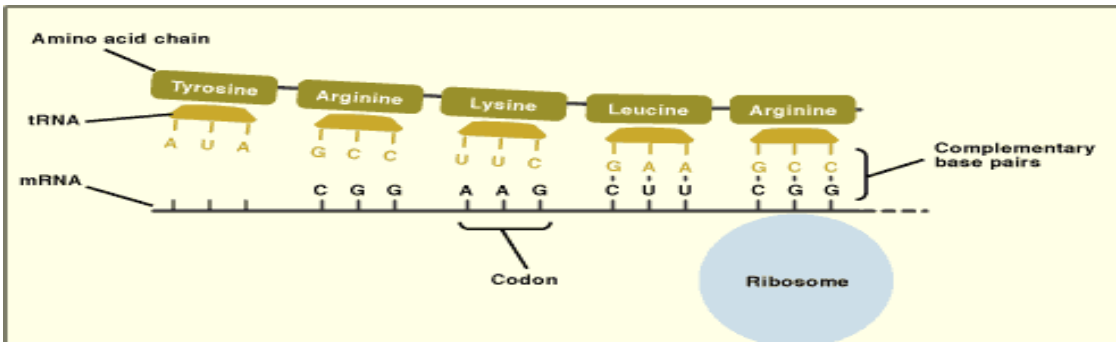
Initiation – RNA polymerase identifies where to begin transcription.

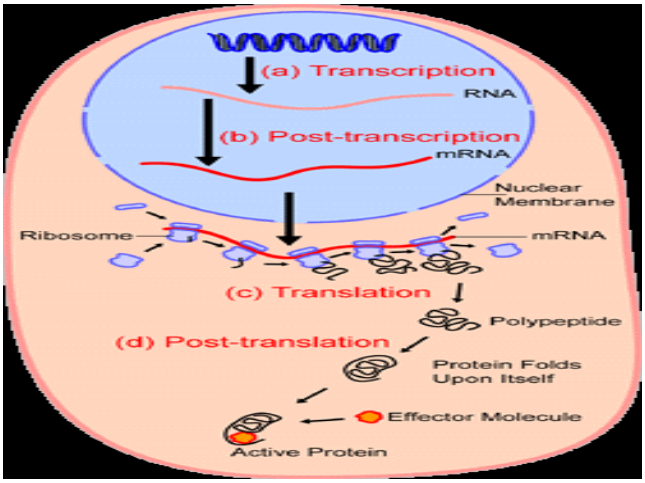
Elongation – RNA nucleotides are added to the 3’ end of the new RNA.

Termination – RNA polymerase stops transcription when it encounters terminators in the DNA sequence.



Translation proceeds through:
Initiation – mRNA, tRNA, and ribosome come together.
Elongation – tRNAs bring amino acids to the ribosome for incorporation into the polypeptide.
Termination – ribosome encounters a stop codon and releases polypeptide.





The main steps from gene to protein.

Concept 1: The Central Dogma

DNA → RNA → protein.

Concept 2: Transcription and Translation in Cells

Transcription and translation are spatially and temporally separated in eukaryotic cells; that is, transcription occurs in the nucleus to produce a pre-mRNA molecule.

The pre-mRNA is typically processed to produce the mature mRNA, which exits the nucleus and is translated in the cytoplasm.

Concept 3: Different Genes for Different RNAs

There are 4 types of RNA, each encoded by its own type of gene.

- mRNA - Messenger RNA: Encodes amino acid sequence of a polypeptide.
- tRNA - Transfer RNA: Brings amino acids to ribosomes during translation.
- rRNA - Ribosomal RNA: With ribosomal proteins, makes up the ribosomes, the organelles that translate the mRNA.
- snRNA - Small nuclear RNA: With proteins, forms complexes that are used in RNA processing in eukaryotes. (Not found in prokaryotes.)

Concept 4: Basic Structure of a Protein-Coding Gene

The promoter is a base-pair sequence that specifies where transcription begins.

The terminator is a sequence that specifies the end of the mRNA transcript.

Concept 5: The RNA Molecule

RNA is structurally similar to DNA.

Concept 6: The Transcription Process

RNA synthesis involves separation of the DNA strands and synthesis of RNA molecule in the 5' to 3' direction by RNA polymerase, using one of the DNA strands as a template.

In complementary base pairing, A, T, G, and C on the template DNA strand specify U, A, C, and G, respectively, on the RNA strand being synthesized.

Concept 7: Complete Transcription of an RNA Molecule

Transcription begins at the promoter, proceeds through the coding region, and ends at the terminator.

Concept 8: mRNA in Prokaryotes

The sequence of a prokaryotic protein-coding gene is colinear with the translated mRNA; that is, the transcript of the gene is the molecule that is translated into the polypeptide.

Concept 9: mRNA in Eukaryotes

The sequence of a eukaryotic protein-coding gene is typically not colinear with the translated mRNA; that is, the transcript of the gene is a molecule that must be processed to remove extra sequences (introns) before it is translated into the polypeptide.

Most eukaryotic protein-coding genes contain segments called introns, which break up the amino acid coding sequence into segments called exons.

The transcript of these genes is the pre-mRNA (precursor-mRNA).

The pre-mRNA is processed in the nucleus to remove the introns and splice the exons together into a translatable mRNA. That mRNA exits the nucleus and is translated in the cytoplasm.

Concept 10: Pre-mRNA Processing (Splicing)

- The intron loops out as snRNPs (small nuclear ribonucleoprotein particles, complexes of snRNAs and proteins) bind to form the spliceosome.
- The intron is excised, and the exons are then spliced together.
- The resulting mature mRNA may then exit the nucleus and be translated in the cytoplasm.

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Third Question : (20 Marks)

(1): Compare between Three only:

1.(RNA Polymerases - DNA Polymerases)

RNA polymerase I transcribes rRNA.
RNA polymerase II transcribes mRNA and some snRNA**.**
RNA polymerase III transcribes tRNA and some other small RNAs**.** Each RNA polymerase recognizes its own promoter.
DNA polymerase I Degradation or Polymerization
DNA polymerase II Excision repair
DNA polymerase III reverse transcriptase**.**

2. (A constitutive gene – A housekeeping gene)

A constitutive gene is a gene that is transcribed continually as opposed to a facultative gene ,which is only transcribed when needed.
– **A housekeeping gene** is a gene that is required to maintain basic cerllular function and so is typically expressed in all cell types of an organism.

3..(Regulator, Structural and Operator genes)

Regulator gene *regulate the action of operator genes and regulate the transcription of structural gene.*
, ***Structural genes*** *responsible on special construction of protein or enzyme protein.*

Operator gene: genes which control switch on ,swith off the structural genes.

3. **(Lac operon and Trp operon in prokaryote.)**

<u>The lactose operon (Lac operon</u>)

The lactose operon contains 3 genes, Lac Z, Y and A. These genes encode for enzymes required to metabolize lactose --> beta-galactosidase, lactose permease, and beta-galactoside transacetylase.

In the absence of lactose:no transcription

In the presence of lactose: yes transcription ……..etc

<u>The Tryptophan operon (Trp operon)</u>

It includes 5 genes involved in Tryptophan synthesis

The genes are expressed as a single mRNA strand, transcribed from an upstream promoter

If tryptophan is lacking, the ribosome will be stalled as it tries to translate the coding region

If tryptophan is present it will bind the trp repressor. This enables it to bind the operator and block the RNA polymerase.

<u>(2): Complete The followings: (Only five Points)</u>

(1): Cytoplasm cytoplasm.

(2) :Carries the genetic information's ,Replications ,encoding information ,mutation recombination and gene expression.

(3): Sanger dideoxy , Scalble.

(4) :Operator

(5): mRNA , Amino acids ,Protein.

(6): bind RNA polymerase to the promoter and initiate transcription

(7) :Isolation of vector and gene source DNA ,Insertion of DNA into the vector . Introduction of the cloning vector into cell, Cloning of cells and foreign genes, and identifying cell clones with the right gene.

(8): Triplet codon, non-overlapping, commaless , non-ambiguous, has polarity , degenerate and universal .

With my best wishes

Prof .Dr / Mohamed Serag Eldin